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ABSTRACT

The primary goal of this application is to develop and evaluate a novel therapeutic approach for the restoration of dystrophin levels in Duchenne Muscular Dystrophy (DMD) patients using recombinant adeno-associated virus (rAAV) vector-mediated gene transfer. More specifically, we propose to evaluate the clinical outcome of intrapleural administration of a Herpes Simplex virus type I System (HSV) production system-derived rAAV serotype 9 (rAAV9) vector encoding a codon-optimized mini dystrophin gene (miniDys) in the golden retriever muscular dystrophy (GRMD) model. This is a logical progression from the culmination of preclinical and clinical work done by our team in the field of gene therapy and myopathies. In particular, we have been able to show that rAAV-mediated gene transfer can result in significant therapeutic transgene expression in both the murine and canine models of muscular dystrophy, including DMD, which have led to current ongoing clinical trials. Furthermore, we demonstrated the superiority of rAAV serotype 9 to confer lasting and elevated gene expression in cardiac and skeletal muscles in murine, canine, and rhesus monkey models, suggesting the feasibility of using this vector system in the human population.

This proposal comprises two specific aims. The first specific aim is to develop a clinically scalable and flexible platform of vector production based on the HSV system originally developed by Byrne *et al*. To-date, large-scale rAAV manufacturing using transfection of adherent cells is extremely cumbersome and time-consuming and generally impractical and unfeasible for large preclinical studies or clinical trials in patients with inherited myopathies. We believe the scalable HSV platform is required for generating sufficient amounts of highly pure and concentrated rAAV9-miniDys necessary for clinical trials. For the proposed studies (and looking toward the future for clinical studies), we have incorporated a codon-optimized version of miniDys to maximize therapeutic effect while minimizing exposure to capsid protein or excess genome copies. In addition, the human Desmin gene promoter (DES) will be used to drive the codon-optimized miniDys cDNA expression in a tissue-restricted manner. At this time, we have created a truncated Desmin promoter to drive the codon-optimized miniDys cDNA. Moreover, the HSV production system has been successfully implemented in our laboratory and, based current yields, we are confident that sufficient material required for the canine studies can be generated in the given time frame.

In the second specific aim (next award period), we will determine if intrapleural delivery of HSV-AAV9-miniDys will augment cardiorespiratory function in the GRMD model. We hypothesize that use of a HSV system produced rAAV9 coupled with an innovative delivery route (intrapleural) can target and correct the most critical tissues (myocardium and respiratory muscles), which affect lifespan in DMD patients. Juvenile GRMD dogs will be administered a single dose of vector to the intrapleural space and serially assessed for respiratory function (at resting and ventilatory challenge), cardiac measurements (ventricular size, ejection fraction, etc.), immune response to vector and therapeutic transgene product, and at study end, miniDys expression. To our knowledge, this is the first study to simultaneously evaluate cardiac and respiratory

outcome measures in a large animal model of muscular dystrophy using a clinically scalable and feasible (HSV) rAAV production method for muscular dystrophy.

Ultimately, our goal is to meaningfully improve the quality of life and extend the life expectancy for patients with DMD. The use of mechanical ventilation further promotes respiratory muscle weakness and directly increases the burden of care and erodes the quality of life for patients. We believe the fulfillment of this proposal would have a two-fold impact on individuals diagnosed with DMD. First, the development of an improved manufacturing method of vector in which large amounts of vector with high purity and potency will be manufactured in a more cost-effective and timely fashion, will ultimately shorten the timeframe to translate the results of this proposal to human clinical trials. Second, delivery of this HSV-AAV9-miniDys via the intrapleural route will provide a novel therapeutic platform for treating respiratory muscles (i.e. diaphragm and accessory respiratory muscles) and the myocardium, which we hope will be a stepping-stone to preventative or restorative treatments for patients with DMD.

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1. INTRODUCTION:

The central objective of this work is to construct a scalable and cost-effective recombinant AAV vector serotype 9 delivery system for the treatment of cardiorespiratory dysfunction in Duchenne Muscular Dystrophy.

2. KEYWORDS:

Duchenne Muscular Dystrophy, AAV, HSV, respiratory, cardiac, miniDystrophin

3. OVERALL PROJECT SUMMARY:

Current objectives:

Aim 1. The overall objectives of this Aim are to 1) develop and implement the HSV production system for the production of rAAV9-miniDys required in Aim 2. Summary of accomplishments and results is provided in this report according to the original task list.

Summary of results:

During year 1, we have further developed all the protocols and reagents needed for the production of rAAV by the HSV system. Figure 1 shows a simplified scheme of the production system. Further details can be found in Clement *et al*., 2009(1). The two critical reagents used to prepare rAAV by HSV coinfection are the recombinant HSV carrying the AAV cassette (ITR – promoter – gene of interest) and the AAV helper genes, *Rep* and *Cap* ORFs. In this specific work we generated rHSV for the AAV Rep2/Cap9 cassette and for the tDes-miniDys AAV construct. A list of all protocols and reagents prepared during this period is provided in Table 1.

Task 1. Implementation of the HSV system for the production of AAV9-miniDys.

1a/b. Plasmid pHSV106-AAV9 carries the AAV2 P5 and Rep sequences, and the AAV9 Cap sequences inserted into the TK ORF of HSV-1. Plasmid was sequenced and sequence identity was confirmed. The AAV vector construct was first generated into the traditional pTR-UF plasmid backbone. Briefly a truncated version of the human Desmin promoter was engineered to allow subcloning of the mini-Dys ORF into an AAV genome. Indeed, the size of the miniDYS is about 3.8 kb and the maximal size of an AAV genome is around 5Kb to allow for optimal packaging. Therefore we deleted about 100 bp from the original Desmin promoter sequence by promoter mapping based on Paulin *et al.'s* work(2). We also decided to use a short poly Adenylation signal sequence of 50 bp based on Flotte *et al* (3). The DNA fragment containing the truncated Desmin promoter (tDes), the codon optimized miniDys and the short polyA was then subcloned into pTR-UF backbone to restore AAV ITRs (Fig 2). This

1. Protocols	
Task	**Status**
V27 cells	
Cultured	*Complete*
Transfected	*Complete*
HSV Plaque Assay	*Complete*
rHSV	
Serial Plaque Isolation	*Complete*
Seed stocks prepared	*Complete*
HSV DNA from infected cells prepared	*Complete*
Western Blot for the detection of AAV9 capsid proteins	*Complete*
2. Reagents	
Task	**Status**
V27 Working Cell Bank	*Complete*
d27.1 HSV DNA from infected cells	*Complete*
pHSV106-AAV9 shuttle plasmid	*Complete*
rHSV-AAV9 Seed Stocks	*Complete*
rHSV-UF5 (GFP) Seed Stocks	*Complete*
pTR-tDes-miniDys	*Complete*
HSV-rAAV9-tDes-miniDys	*In progress*

Table 1. List of protocols and reagents developed for Aim 1.

construct was then tested *in vitro* (HEK293 – Figure 3, C2C12 data not shown, C12 cell lines data not shown) prior to be subcloned into the HSV shuttle plasmid pHSV106.

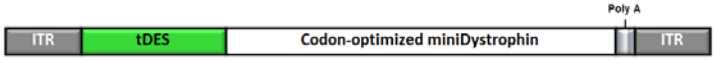

Figure 2. Elements of pTR-miniDys plasmid.

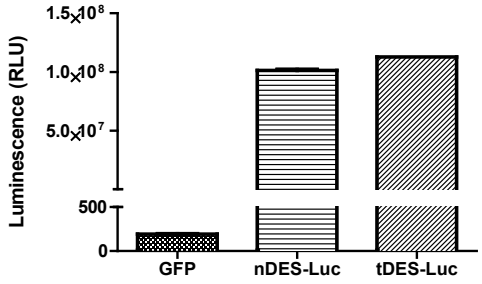

Figure 3. Comparison of a plasmid containing full length or a truncated desmin promoter for luciferase expression. Assessment of 293 cell lysates for luciferase activity demonstrates similar expression activity for each DES promoter.

Conclusion: Assessment of transduction and expression of truncated-Desmin driven mini-dystrophin following administration of transfection based rAAV-tDes-miniDys is ongoing in a murine model (MDX) of DMD and is near completion (expected completion date: 10/20/2014).

1c/d. Generation of rHSV-AAV9. Recombinant HSV-AAV9 was generated by homologous recombination after transfection of plasmid pHSV106-AAV9 together with HSV-UF5 DNA in V27 cells (6-well plate)(4). Several clear plaques were isolated by three rounds of infection on V27 (10cm dish). Plaques were tested by PCR to confirm presence of the AAV cassette (primer at the junction AAV2 Rep - AAV9 Cap) and two clonal plaques were further amplified into T-225 flasks to generate material for small-scale rAAV9 production (see below). *Generation of rHSV-AAV-tDes-miniDYS is currently in progress.*

1e. Preparation of rHSV-AAV9 Seed Stocks. Seed stocks of rHSV-AAV9 were prepared in cell factories (Corning, CellSTACK®). Briefly ~ 1E+09 V27 cells were infected with HSV-AAV9 prepared in 1d. at an MOI of ~ 0.1 (see Table 2). Cells, media or cells and media combined were harvested at 72 hrs, clarified by centrifugation and stored frozen. Current protocol is based on an *in situ* cell lysis by adding ~ 0.6M final NaCl to the cell media and incubation for ~ 30 min. Cell debris are then removed by centrifugation and 5% final glycerol added for storage. Seed stocks of HSV-GFP were also prepared. This HSV carries the AAV construct with CMV-GFP and was used in pilot runs to establish AAV9 production conditions in 293 cells, as described below. A summary of the seed stocks production is provided in Table 2.

AAV9 production Pilot runs. Seed stocks of rHSV stocks shown in Table 2 were used to produce rAAV9-GFP by coinfection in 293 cells seeded in T225 flasks ($2x10^7$ cells) with HSV-AAV9 and HSV-UF5 (GFP) at various MOIs. Coinfections were performed

Lot #	*HSV9-081814*	*HSV9-071114*	*HSV9-051614*	*HSV-GFP-072514*	*HSV-GFP-051614*
Input (total pfu)	1.50E+08	3.80E+07	1.12E+08	1.50E+08	1.00E+08
MOI	~ 0.15	~ 0.08	~ 0.11	~ 0.15	~ 0.1
Final Titer (pfu/mL)	1.50E+07	1.90E+07	1.50E+07	5.25E+07	1.46E+07
Formulation	NaCl/Glycerol	NaCl/Glycerol	DMEM/DPBS	NaCl/Glycerol	DMEM/DPBS
Volume (mL)	1250	1250	1135	1300	1110
Total pfu	**1.88E+10**	**2.38E+10**	**1.70E+10**	**6.83E+10**	**1.60E+10**
Amplification factor	125	625	152	455	160
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Table 2. Summary of recombinant HSV Seed Stocks.

side-by-side with transfection of plasmid pTRUF5 (AAV-GFP) and AAV9 helper (pXYZ9) according to our standard protocol. Cells were harvested at ~ 52 hours post-infection and 72 hrs post-transfection and AAV9 was purified according to our standard procedure, Benzonase (Crude lysates), followed by iodixanol. Stocks were tested for vector genome by Real-time Q-PCR, infectious titers by Green Cell Assay, and amounts of capsid proteins by Western Blotting using B1 antibody (Progen Biotechnick, Holland). Results are summarized in Figure 3. Vector genome titers showed that when using optimal conditions of HSV-GFP at an MOI of 2 and HSV-AAV9 at an MOI of 12, vector production was increased by up to 6 fold compared to transfection (Fig 3A). Higher MOIs of the HSV-GFP, or lower MOIs of the HSV-AAV9 resulted in similar or lower yields (data not shown). More interestingly, the infectious titers based on GFP-expressing cell counts, revealed that infectious titer was increased by 10 (crude lysates, not shown) to up to 28 fold as compared to rAAV9 generated by transfection (Fig 3B). This resulted in particle-to-infectivity ratios lowered by approximately 6 fold, suggesting that particles generated by the HSV system are more infectious. Importantly this increase in infectivity is not linear with the increase in capsid protein amount, in that western blot and western blot quantification analysis reveal that the amount of capsid is not proportional to the increase in particle titers, suggesting that less particles may be produced with better potency (data not shown).

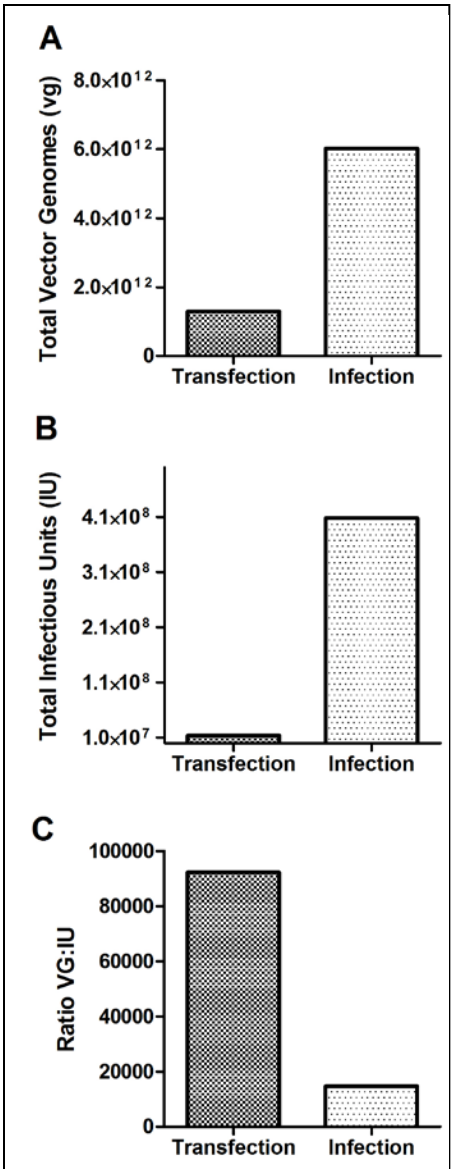
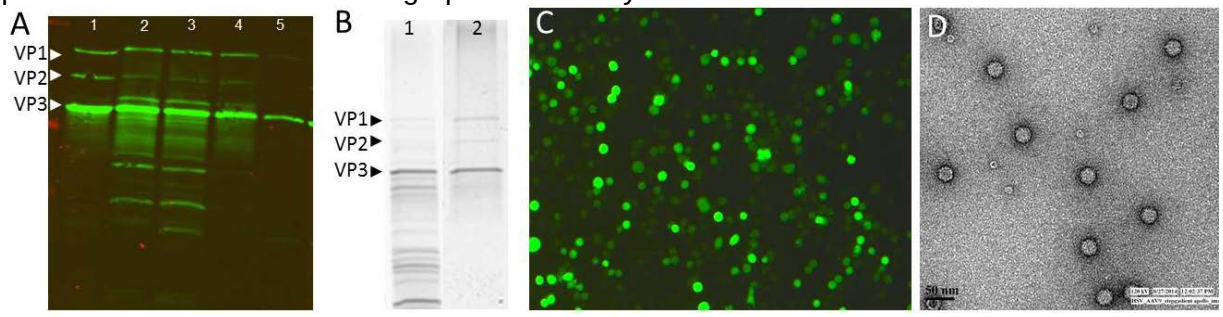


Figure 3. Titers of rAAV9-GFP from iodixanols purified stocks made by transfection (Gray bars) or HSV coinfection (clear bars). A: Total vector genomes per flask; B: total infectious units per flask; C ratio vector genome to infectious unit.

Large Scale AAV9 production. One pilot large-scale production was performed in one CellStack with ~ 1E+9 HEK293 cells. Cells were coinfected with rHSV-GFP and rHSV-AAV9 Seed Stocks at ~ 1 pfu/cell (MOI of 1) in serum free DMEM for 1 hour. It is noteworthy that optimal MOI of 2 and 12 with the HSV-GFP and HSV-AAV9 respectively could not be achieved in this pilot run. Media was then changed and replaced with 5% serum DMEM and cells were incubated for approximately 50-52 hours. At time of harvest, media was discarded, and 293 cells were washed with 1X DPBS and detached with PBS/EDTA, spun down and homogenized in 60 mL of Lysis Buffer (Tris/NaCl). AAV9 particles were purified from the crude by column chromatography based on the method described in Potter *et al.* 2014 (in press), developed in our lab specifically for AAV9. Briefly, after addition of sodium citrate and

Figure 4. Quality Control Evaluation of HSV-made rAAV9. A. Western Blot. Vector was made by HSV unless otherwise specified. Lane 1. AAV9-GFP Crude Lysate; Lane 2: AAV9-GFP Purified bulk, 2.5E+10 vg; Lane 3: AAV9-UF11 purified bulk made by transfection, 2.5E+10 vg; Lane 4: AAV9-GFP Final Stock, 2.5E+10 vg; Lane 5: AAV9-GFP Final Stock, 1.6E+10 vg. **B. Silver stained gel.** 10% SDS-PAGE. Lane 1 - Purified bulk (3.6E+10 vg) and lane 2 - final stock (3E+10 vg) **C. Infected cells.** C12 cells infected with rAAV9-GFP purified bulk. **D.** Electron-micrograph obtained by TEM from AAV9-GFP final stock.



citric acid to the crude, benzonase digestion and clarification by centrifugation, the virus-containing sample was loaded onto an SP column (SP, GE Healthcare) and eluted by salt gradient. The Purified Bulk was eluted as a single ~ 29 mL fraction and stored frozen for QC assessment. A third of the SP bulk was processed over iodixanol gradient followed by concentration by centrifugal device (Apollo, Orbital Sciences) and buffer exchanged in Lactated Ringer's solution. This final stock was assessed for vector genome titer by Q-PCR, infectious titer by infectious center assay (Desmin ^{32}P probe) or Green Cell Assay (Fig 4C), identity by Silver stained gels (Figure 4B), and total capsid amounts estimated by Western Blot (Figure 4A, B1 Antibody). Results are summarized in Table 3 and Figure 4.

Assay	Purified Bulk	Final Stock*
Vector Genome Titer	$1.2x10^{12}$ vg/mL	$1x10^{12}$ vg/mL
Infectious Titer	$1.35x10^{8}$ iu/mL	$2x10^{7}$ iu/mL
Volume	29 mL	2 mL
Total vg*	$3.48x10^{13}$ vg	$2x10^{12}$ vg
Total iu	$3.9x10^{9}$ iu	$4x10^{7}$ iu
Ratio vg: iu	$8.92x10^{3}$	$5x10^{4}$
Yield by CF	$3.48x10^{13}$	$6x10^{12**}$

Table 3. Quality Assessment of AAV9 stocks.* Represent 1/3 of the total PB. ** Extrapolated by multiplying yield by 3.

Overall results showed that production of AAV9-GFP from one cell factory of 293 cells resulted in yield comparable to our standard AAV9-GFP yield obtained by transfection, despite using under optimal MOIs. Further, the virus' increased potency still holds true for the SP bulk with a ratio < 1E+4. The potency appeared to decrease when testing the final stock, but this is currently under further evaluation. The loss of viral material observed during the iodixanol and concentration processes are also routinely observed in transfection-made AAV9. Semi-quantification of the amount of capsids was performed by loading similar amount of vector genomes for the HSV-made preparations side by side with a transfection-made one, before (purified bulks) and after iodixanol gradient purification (final stocks) on protein gel. Preliminary Western data suggest that the amount of total capsids is relatively similar with both methods (figure 3A). A micrograph obtained by Transmission electron-micrography (kindly performed by the

laboratory of Dr. Agbanje-McKenna, UF) suggest a ratio of full to empty around 4 fulls for 1 empty, after iodixanol gradient purification.

Although very encouraging, subsequent testing using optimal MOIs defined in the small scale experiment are underway. Extrapolation of the small-scale data suggest that one may expect to reach ~ $1.7x10^{14}$ vg per CellStack in purified bulks.

Accomplishments, Discussion and Future Directions for Aim 1

Current yields based on pilot runs using and HSV containing AAV-GFP show that an average 1 – 2E+14 vg can be obtained for each Cellstack of HEK293. We are currently optimizing several steps of the overall production and purification processes: 1) we will be using tangential flow filtration (TFF) as a final polishing and concentrating step, that currently results in > 80% recovery of the purified bulk material; 2) We will be further concentrating our HSV Seed stocks material by TFF to obtain workable volume for infection at large scale; and 3) we are setting up culture of suspension adapted BHK21 cells in spinner flasks. It has been reported that yields obtained from BHK21 are further enhanced while MOI of HSV inputs are reduced.

The method improvements will be used to produce the rAAV9-tDes-miniDys stocks in amount sufficient to support Aim 2 of this work.

Specific Aim 2: To determine if intrapleural delivery of HSV-AAV9-miniDys will augment cardiorespiratory function in a canine model of muscular dystrophy (GRMD).

2a/b. Procure a total of 9 GRMD dogs, divided into two groups of 5 treated (HSV-rAAV9-miniDys) and 4 untreated GRMD controls. We have worked closely with TAMU PI: Joe Kornegay and testing (cardiac/respiratory parameters) of carriers have been ongoing in mid-late quarters of year 1. Breeding is planned to coincide with production of HSV-AAV9 material as scheduled.

3/4. In coordination with TAMU Subcontract PI: Joe N. Kornegay, DVM, PhD, we have completed regulatory and approval processes for experiments in GRMD animals (task 3). Cardiac MRI protocols for the GRMD have been developed and/or optimized to determine the progression of cardiomyopathy and potential for efficacy following AAV9 vector administration. Protocols include the following: 2ch/4ch, left ventricle short axis, aortic flow quantification, pre- and post-contrast Modified Look-Locker Inversion Recovery (MOLLI), and 2ch/4ch multiple left ventricle short axis with gadolinium delayed enhancement. Briefly, these measures will allow us to quantify ejection fraction, stroke volume, cardiac output, end diastolic/systolic volume, aortic flow, and fibrosis in the canine heart. In figure 5, pre-contrast MOLLI detected elevated T1 relaxation time when performed on young (3 months old) GRMD and carrier animals. Although preliminary, these results suggest the development of fibrotic lesions is present at an early time-point in the model.

In respect to respiratory functional assessments (spirometry and respiratory inductive plethysmography), we have completed a two day on-site (TAMU) training course with Data Sciences International. At this time key personnel are trained for respiratory measures and characterization of respiratory function is underway on existing carrier animals.

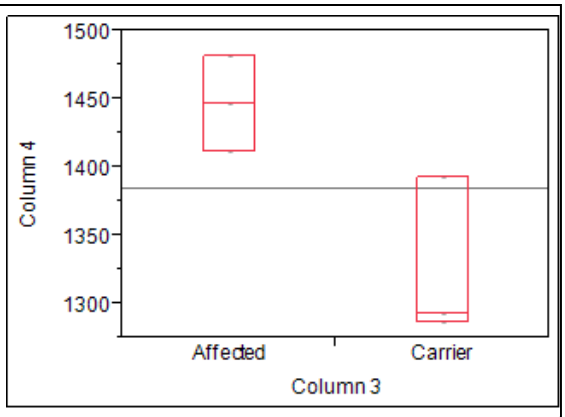


Figure 5. Detection of fibrotic lesions in GRMD. Pre-contrast MOLLI LV basal T1 relaxation time is significantly higher in GRMD compared to carriers at 3 months of age. This may suggest the presence of fibrotic lesions in the left ventricle of affected animals.

4. KEY RESEARCH ACCOMPLISHMENTS:

- Demonstration of superior vector genome **:** infectious unit ratio with the HSV method.

5. CONCLUSION: To date, we have successfully created a construct to drive the expression of a human codon-optimized mini-dystrophin with a truncated desmin promoter. In previous studies with an alternative disease model of cardiorespiratory dysfunction (Pompe disease), we have shown the Desmin promoter to drive high transgene expression resulting in improved cardiac and respiratory function and morphology. We anticipate this promoter to perform in a similar fashion in the GRMD model. In addition, we have created HSV-rAAV9 seed stocks that will allow investigators the opportunity to utilize a scalable AAV production method. Our results suggest HSV-AAV has a significantly lower vector genome : infectious unit ratio when compared to transfection-AAV preparations. In essence, we hope the HSV-AAV methodology will allow for production of viral vector preparations at large scale and that the preparations will have a higher infectivity. The combination of HSV/AAV desmin-miniDystrophin may lead to improved efficacy in respect to cardiac and respiratory dysfunction in the canine model of DMD.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS: in preparation - nothing to report.

7. INVENTIONS, PATENTS AND LICENSES: nothing to report.

8. REPORTABLE OUTCOMES: Provide a list of reportable outcomes that have resulted from this research. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. This list may include development of prototypes, computer programs and/or software (such as databases and animal models, etc.) or similar products that may be commercialized.

- Creation of the first recombinant AAV construct with a truncated Desmin promoter to drive expression of mini-dystrophin.
- Creation of the first AAV vector preparation containing a truncated Desmin promoter to drive expression of mini-dystrophin.

- Creation of the first HSV/AAV construct with a truncated Desmin promoter to drive expression of mini-dystrophin.

9. OTHER ACHIEVEMENTS: This list may include degrees obtained that are supported by this award, development of cell lines, tissue or serum repositories, funding applied for based on work supported by this award, and employment or research opportunities applied for and/or received based on experience/training supported by this award.

- Preparation of rHSV-AAV9 Seed Stocks

For each section, 4 through 9, if there is no reportable outcome, state "Nothing to report."

10. REFERENCES CITED:

1. Clement N, Knop DR, Byrne BJ. Large-scale adeno-associated viral vector production using a herpesvirus-based system enables manufacturing for clinical studies. Hum Gene Ther. 2009;20(8):796-806. PMCID: 2861951.
2. Paulin D, Li Z. Desmin: a major intermediate filament protein essential for the structural integrity and function of muscle. Experimental cell research. 2004;301(1):1-7.
3. Flotte TR, Afione SA, Solow R, Drumm ML, Markakis D, Guggino WB, et al. Expression of the cystic fibrosis transmembrane conductance regulator from a novel adeno-associated virus promoter. J Biol Chem. 1993;268(5):3781-90.
4. Conway JE, Rhys CM, Zolotukhin I, Zolotukhin S, Muzyczka N, Hayward GS, et al. High-titer recombinant adeno-associated virus production utilizing a recombinant herpes simplex virus type I vector expressing AAV-2 Rep and Cap. Gene Ther. 1999;6(6):986-93.

11. APPENDICES: n/a